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TRANSLATIONAL MEDICINE: BENCH TO BEDSIDE

Recent Advances in Celiac Disease from TTG to Gluten in Pee

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The gluten-free diet can be life saving for individuals with celiac disease, yet adherence to this difficult treatment is poor and long-term outcomes are suboptimal with increased risk of complications, including lymphoma. Many fundamental issues relating to the pathogenesis and treatment of celiac disease remain unsolved. Celiac disease occurs exclusively among HLADQ2 and HLADQ8-positive individuals, yet what leads to the loss of oral tolerance to gluten remains an enigma. Two recent rigorously conducted prospective birth cohort studies of genetically susceptible individuals[1,2] and a randomized feeding intervention[3] did not demonstrate an association between the development of CD and breastfeeding, or age at gluten introduction—each of which had been implicated in prior epidemiologic studies.[4] In contrast, several recent laboratory discoveries have identified potential therapeutic targets for celiac disease, as well as an alternate method for monitoring adherence to a gluten-free diet. In this review, we will discuss five approaches delineating the molecular pathophysiology of celiac disease, which have the potential to improve celiac disease treatment and management in the coming years.

Structural studies of the interactions between: (1) the T-cell receptor (TCR) and HLADQ2 (ref. 5) and (2) anti-tissue transglutaminase autoantibodies and tissue transglutaminase-2 (TTG-2)[6] suggest potential additional molecular determinants of celiac disease autoimmunity. Petersen *et al.*[5] used X-ray crystallography to determine the structure of four different TCRs in complex with HLADQ2 bound to one of two immunodominant deamidated gliadin peptides (glia-α1a or glia-α2). These TCRs from different individuals demonstrated a striking structural similarity. A conserved arginine residue at position 109 of the hypervariable complementarity determining region (CDR3) of the TCR beta chain has a critical role. This arginine residue acts as a linchpin stabilizing the structure through interactions with the gliadin peptide as well as both the alpha and beta chains of HLADQ2. In addition, interactions between HLADQ2 and asparagine 36 tyrosine 38 motifs in CDR1 of the TCR alpha chain suggest a structural basis for the observed limited T-cell repertoire. The preferential selection of αβ T cells may be a defining feature of celiac disease as this has also been observed for HLADQ8.[7]

An analogous biased usage of both heavy- and light-chain genes in anti-tissue transglutaminase antibodies has also been reported.[8] Curiously, attempts to cocrystallize TTG-2 with a prototype antibody directed to TTG-2 epitope 1 derived from a patient with celiac disease were unsuccessful.[6] Therefore, Chen *et al.*[6] determined the structure of the Fab fragment in isolation and used molecular modeling of potential interactions with TTG-2 in isolation[9] to guide site-directed mutagenesis of TTG-2. Small-angle X-ray scattering (SAXS) and biochemical methods were used to characterize the interaction between the antibody and TTG-2 epitope 1. In their model, a salt bridge between lysine 82 of the framework region of the heavy chain and aspartate 191 in the TTG-2 active site provides a structural basis for observed allelic specificity. The orientation of the immunoglobulin towards the active site further suggests that TTG-2 may have a mechanistic role through cross-linking of B-cell receptors, thus explaining the high frequency of antibodies directed against TTG-2 epitope 1 in patients with celiac disease.

The requirement for a genetically susceptible individual to produce a TCR with specific alpha and beta chains coupled to a CDR that recognizes gluten bound to the major histocompatibility complex and generate a B cell through VDJ recombination of specific immunoglobulin heavy and light chains with a hypervariable region that recognizes TTG-2 may partially explain the incomplete penetrance of celiac disease. If HLADQ2/DQ8-positive persons who do not produce such T cells and immunoglobulins do not develop celiac disease, then this might be used to improve upon current genetics-based risk stratification algorithms.

Environmental factors likely also have a role in the loss of tolerance to gluten. The discovery that interleukin (IL)-15 induces a T_H1 response in the presence of other proinflammatory mediators (i.e., retinoic acid and IL-12) implicates the innate immune system in the loss of oral tolerance to gluten.[10] In murine models, gliadin-fed humanized DQ8-D^d-IL-15 mice, which constitutively overexpress IL-15 in the lamina propria, developed intraepithelial lymphocytosis and anti-gliadin and anti-TTG antibodies without villous atrophy, which is characteristic of mild enteropathy celiac disease. Others have shown that in T3b-hIL-15 transgenic mice (which hyper-express human IL-15 using an enterocyte-specific promoter) villous atrophy can be reversed by tofacitinib, a janus kinase inhibitor that interrupts IL-15 signaling.[11] Therapeutic modulation of IL-15 signaling is an area of intense investigation.

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Clinical trials of Hu-Mikβ1 in refractory celiac disease type are actively recruiting. Clinical response to this CD122 (IL-2/IL-15Rbeta) targeted monoclonal antibody was not observed in Phase I trials for T-cell large granular lymphocytic leukemia.[12] Trials of other agents that act upon the IL-15 pathway are being planned.

TTG-2 is both the autoantigen in celiac disease and a serine protease that catalyzes the deamidation of gluten peptides, which increases their immunogenicity. Galipeau *et al.*[13] have demonstrated how this might be exploited in the treatment of celiac disease using elafin. This potent serine protease inhibitor is expressed throughout the human gastrointestinal tract with decreased expression in patients with inflammatory bowel disease.[14] Elafin levels are also decreased in celiac disease and elafin inhibits the deamidation of toxic gluten peptides by TTG-2 *in vitro.*[13] The therapeutic potential was then demonstrated in an elegant *in vivo* experiment using a food grade strain of *Lactococcus lactis* to deliver elafin to gliadin-sensitized NOD/DQ8 mice. Treatment with elafin decreased intraepithelial lymphocytosis and normalized intestinal barrier function, which was associated with preserved expression of the tight-junction protein zonula occludens-1.

Until these therapies become widely available, the gluten-free diet remains the only effective treatment for celiac disease. Assessing treatment adherence remains a challenge as there is no practical tool available for routine use.[15] Consequently, practice guidelines for long-term monitoring of patients with celiac disease are vague and supported primarily by expert opinion.[16] Patients often must rely upon symptoms (if they have them) to retrospectively identify gluten consumption. Reports of the successful use of antibodies to detect gluten immunogenic peptides in stool[17] and development of tests for urine[18] represent a paradigm shift away from traditional dietitian-based approaches. These tests exploit the fact that gluten is incompletely hydrolyzed by human endoluminal proteases thus it is excreted intact in feces. They also demonstrate that gluten is systemically absorbed and excreted in urine. Further development of these tools for patient use has the potential to revolutionize how patients approach the gluten-free diet as they can confirm whether gluten exposure has occurred. Point-of-care tools may radically alter how the gluten-free diet is managed while alternative or adjunct treatments to a gluten-free diet are developed.

CONFLICT OF INTEREST

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